AMENDMENT

IN THE SPECIFICATION:

On page 1, line 7-13:

This application is a divisional of U.S. Serial No. 09/397,233, filed September 16, 1999, now U.S. Patent No. 6,630,327, issued October 7, 2003, which is a divisional of U.S. Serial No. 08/752,447, filed November 16, 1996, now U.S. Patent No. 5,994,088, issued November 30, 1999, which is a continuation-in-part of U.S. patent application, Serial No. 08/483,290, filed June 7, 1995, which is a divisional of Serial No. 08/128,056, filed September 28, 1993, now U.S. Patent No. 5,434,075, issued November 7, 1995, which is a continuation of Serial No. 07/666,340, filed March 8, 1991, now abandoned. This application is also a continuation-in-part of USSN 08/482,670, filed June 7, 1995, which is a divisional of Serial No. 08/032,056, filed March 16, 1993, which is a continuation-in-part of 07/854,881, filed March 20, 1992, now U.S. Patent 5,434,075, issued July 18, 1995.

On page 1, line 28:

Many human cancers express intrinsically or develop spontaneously resistance to several classes of anticancer drugs, each with a different structure and different mechanism of action. This phenomenon, which can be mimicked in cultured mammalian cells selected for resistance to certain plant alkaloids or antitumor antibiotics such as colchicine, vinblastine and doxorubicin (~~former~~formerly known as Adriamycin), is generally referred to as multidrug resistance ("MDR"; *see* Roninson (ed)., 1991, Molecular and Cellular Biology of Multidrug Resistance in Tumor Cells, Plenum Press, N.Y., 1991; Gottesman *et al.*, 1991, *in* Biochemical Bases for Multidrug Resistance in Cancer, Academic Press, N.Y., Chapter 11 *for reviews*). The MDR phenotype presents a major obstacle to successful cancer chemotherapy in human patients.

On page 3, line 1:

Expression of Pgp on the cell surface is sufficient to render cells resistant to many (but not all) cytotoxic drugs, including many anti-cancer agents. Pgp-mediated MDR appears to be an important clinical component of ~~tumor~~ drug resistance in tumors of different types, and *MDR*1 gene expression correlates with resistance to chemotherapy in different types of cancer.

On page 13, line 2:

Figures 1A through 1H~~Figure 1~~ depicts the predicted nucleic acid sequence of human Pgp (Seq. I.D. No.1), wherein the initiation (ATG) and termination (TGA) codons, as well as codons encoding mutations at amino acid positions 433 and 1076, are highlighted.

On page 13, line 18:

Figures 6A through 6D illustrates flow cytometric analysis of mouse L cell transfectants expressing wildtype (KK-L; Figures 6A and 6B), or double mutant (MM; Figures 6C and 6D) human Pgp incubated with PE-conjugated UIC2 ~~(Figures 6A and 6C)~~ (Figures 5A and 5C) or MRK16 (Figures 5B and 5D) ~~(Figures 6B and 6D)~~ in the presence of absence of taxol, vinblastine or etoposide.

On page 14, line 3:

Figures 9A through ~~9E~~ 9C illustrate flow cytometric analysis of K562/i-S9 leukemia cells incubated with PE-conjugated UIC2 in the presence or absence of vinblastine and varying concentrations of the ATP depletion agents oligomycin, azide and cyanide.

On page 16, line 15:

In particular, the invention provides methods and immunological reagents for purifying hematopoietic stem cells from a biological sample, such as bone marrow or human cord blood. As provided by the invention such methods include conventional cell separation methods and techniques, including but not limited to fluorescence activated cell sorting techniques, and more specifically to the use of immunomagnetic beads and immunoaffinity columns for achieving cell separation. It will be recognized that in the practice of the methods of the invention using these separation techniques, the immunological reagents of the invention are provided in certain embodiments conjugated to immunomagnetic beads or immunoaffinity columns, to achieve separation of hematopoietic stem cells based on expression of Pgp. In other embodiments, the methods of the invention are provided wherein the immunological reagents of the invention are recognized by immunomagnetic bead-conjugated or immunoaffinity column-conjugated second immunological reagents which specifically recognize the immunological reagents of the invention (for example, based on isotypic, allotypic or species-specific antibodies or antisera). Also encompassed within the immunological detection methods useful in the practice of the invention are immunological reagents labeled with a fluorescent label and separation of the hematopoietic cells reacted with the fluorescently-labeled immunological reagent from other cells in the biological sample by fluorescence activated cell sorting. Alternative separation methods depending on specific and enhanced recognition of Pgp using the immunological reagents of this invention will be understood by those with skill in the art and are encompassed within the methods of the invention.

On page 18, line 9:

Such cells were used to immunize syngeneic mice. Appropriate numbers of cells were injected subcutaneously (s.c.) or intraperitoneally (i.p.) by art-recognized immunization protocols (*see* co-owned and co-pending U.S. patent application Serial No. 07/854,881, filed March 20, 1992, now U.S. Patent 5,434,075, issued July 18, 1995, and Serial No. 08/032,056, filed March 16, 1993,each of which is ~~are~~ incorporated in their entirety herein). Typically, 10^5 to 10^8 transfected cells were injected 5 or 6 times at two week intervals, and a final boosting was done with, for example, 10^6 cells subcutaneously and/or intravenously. At an appropriate time after the booster injection, typically 3 to 5 days thereafter, the spleen was harvested from a hyperimmune mouse, and hybridomas generated by standard procedures (*see, e.g.*, Kearney *et al.*, 1979, *J. Immunol.* 123: 1548) using human myeloma cells, *for example*, P3-X63-Ag 8.653 (A.T.C.C., Rockville, MD).

On page 19, line 6:

Alternative methods for producing mAbs are known in the art (as described in co-owned and co-pending U.S. patent application Serial No. 07/854,881, filed March 20, 1992, now U.S. Patent 5,434,075, issued July 18, 1995, and Serial No. 08/032,056, filed March 16, 1993, each of which is ~~are~~ incorporated in its entirety herein).

On page 19, line 21:

The immunological reagents of the invention are ~~provides~~ provided to specifically recognize and bind to Pgp, preferably human Pgp, in a conformation adopted in the presence of Pgp substrates, ATP depleting agents, and certain mutant embodiments of Pgp. As used herein, the term "mutant embodiments of Pgp" is intended to encompass heterologous Pgp protein wherein particular amino acid residues in the ATP binding site of each half of the Pgp molecule are altered to provide a mutant or variant Pgp molecule. In preferred embodiments, the heterologous ~~P-glycoprotein expressing-syngeneic~~ P-glycoprotein-expressing syngeneic cells express a mutant P-glycoprotein wherein each of the ATPase-specific active sites carry mutations that prevent ATP binding and/or ATP hydrolysis by these mutant Pgp proteins. In preferred embodiments, such mutants are characterized by amino acid substitution mutations in active site amino acid residues. In certain preferred embodiments, the substituted amino acid residues are lysine residues in the ATPase sites. In other preferred embodiments, the substituted amino acid residues are glycine residues. In particularly preferred embodiments, the mutant the Pgp protein is human Pgp wherein the lysine residues at positions 433 and 1076 of the 1280 Pgp amino acid sequence are substituted with another amino acid, preferably methionine. In other preferred embodiments, the heterologous P-glycoprotein expressing-syngeneic cells express a mutant P-glycoprotein having amino acid substitution mutations at ATPase active site glycine residues, preferably glycine residues at positions 432 and 1075 of the 1280 Pgp amino acid sequence. Preferably, said glycine residues are substituted with serine residues. It will be understood by those with skill in the art that substitutions within the ATP binding sites may involve other amino acid residues or comprise substitutions with other amino acids than the preferred methionine or serine residues explicitly mentioned here. All such ATPase active site mutants of Pgp are intended to fall within the scope of this disclosure.

On page 23, line5:

Monoclonal antibodies specific for human P-glycoprotein were prepared as disclosed in co-owned and co-pending U.S. patent application Serial No. 07/854,881, filed March 20, 1992, now U.S. Patent 5,434,075, issued July 18, 1995, and Serial No. 08/032,056, filed March 16, 1993, each of which ~~are~~ is incorporated in its entirety herein.

On page 24, line 3:

A stable hybridoma line secreting UIC2 mAb was established by three consecutive rounds of subcloning by end-point dilution and screening of the supernatant fluids, as described in co-owned and co-pending U.S. patent application Serial No. 07/854,881, filed March 20, 1992, now U.S.

Patent 5,434,075, issued July 18, 1995, and Serial No. 08/032,056, filed March 16, 1993, each of which ~~are~~ is incorporated in its entirety herein.

On page 26, line 26:

A variety of MDR drugs and competitive inhibitors of Pgp were tested to determine whether these compounds could induce the FACS profile shift observed with UIC2 binding in the presence of vinblastine. The tested compounds included vinblastine, taxol, actinomycin D, gramicidin D, cyclosporine A, reserpine, 5-fluorouracil and methotrexate. The results of these experiments are shown in Figure 3A for binding of PE-UIC2 mAb and in Figure 3B for binding of MRK16 mAb. In these experiments, a rightwards shift in the flow cytometry profile of cells contacted with PE-UIC2 mAb was observed for cells treated with vinblastine, taxol, actinomycin D, gramicidin D cyclosporine A and reserpine. No FACS profile shift was observed in cells treated with 5-fluorouracil or methotrexate, supporting the conclusion that shifting was Pgp specific and was specifically induced with Pgp substrates (since neither ~~to shift~~ 5-fluorouracil or methotrexate is (typically) a Pgp substrate). In contrast, and consistent with the earlier results disclosed above, no change in the flow cytometry profile of cells contacted with MRK16 mAb was observed in cells treated with any of the tested drugs. Stimulation of UIC2 reactivity by these compounds was dose-dependent, for some compounds, while for others no shift was observed at any concentration tested (as illustrated in Figure 4).

On page 27, line 6:

Increased UIC2 reactivity in the presence of Pgp substrates was also observed with other Pgp-expressing cells and cell lines, including PA317 cells expressing Pgp *via* an *MDR*1-encoding ~~retrovirus(Choi *et al., ibid.*),~~ retrovirus (Choi *et al., ibid.*), NIH 3T3 cells, KB-3-1, VSV1 and GSV1 cells transfected with *MDR*1 cDNA (Choi *et al.*, 1988, *Cell* 53: 519-529), Pgp-positive leukemia/lymphoma and tumor samples and normal B- and T-lymphocyte subpopulations and hematopoietic stem cells expressing Pgp (Chaudhary *et al.*, 1992, *Blood ibid.*; Chaudhary *et al.*, 1992, *Cell ibid.*). The concentrations of Pgp substrates producing maximal stimulation of UIC2 reactivity differed slightly for different cell lines and appeared to correlate with the levels of Pgp expressed on the corresponding cell lines.

On page 31, line 17:

The results described in Example 3 above indicated that maximal UIC2 mAb reactivity was associated with the MM mutant, which carries disabling mutations in both nucleotide-binding sites. This result suggested that the biochemical conformation of Pgp that is specifically recognized by UIC2 mAb could reflect a conformation in which Pgp had no bound ATP. This further suggested that intracellular ATP depleting agents would increase UIC2 mAb reactivity for Pgp. Three different agents that induce ATP depletion, sodium cyanide, sodium azide and oligomycin (all of which are specific for mitochondrial enzymes and mechanisms which generate ATP) were used to deplete Pgp-expressing cells of intracellular ATP. All three agents were found to increase UIC2 mAb reactivity to wild-type Pgp in KK-L (Figure 8A) and K562/I-S9 cells (~~Figure 9~~ Figures 9A through 9C). The increase in UIC2 reactivity correlated with the extent of intracellular ATP depletion, as measured by the luciferase assay described in Example 1.

On page 31, line 25:

The addition of cyanide, azide or oligomycin to the series of LM*tk*$^{-}$ cells transfected with different Pgp mutants had the same effect on UIC2 mAb reactivity as the addition of Pgp transport substrates (Figures 8A through 8E). These agents increased the reactivity of KK-L cells to the level of MM (*compare* Figures 8A and 8B), while having no effect on the MM cell reactivity, and increased the reactivity of KK-H, MK-H and KM-H cell lines to similar final levels. Similar results were obtained in K562/I-S9 cells expressing human Pgp (Figures 9A through ~~9E~~ 9C), and a comparison of UIC2 (Figure 10A) and MRK16 (Figure 10B) binding of KK-L cells expressing the wildtype human Pgp is shown in Figures 10A and 10B.